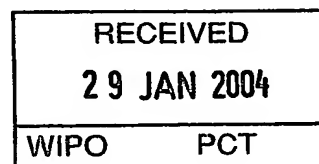


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Pia Høybye-Olsen



Modtaget PVS

27 DEC. 2002

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A sensor and a method of its production*Field of the invention*

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The present invention relates to a sensor comprising one or more sensor units, wherein each sensor units comprises a capture surface area and a piezoresistive detection system, for direct detection of stress change of the sensor unit. One type - the most commonly used type of

10 sensor unit - is a cantilever.

The invention also relates to a method of producing such sensor.

15

Background of the invention

In the art of detecting components in fluids, cantilever based sensors with integrated piezoresistors are used as very sensitive mechanical stress sensors. As described in

20 e.g. WO 0066266 and WO 9938007, micro cantilevers can be used for detection of molecular interaction. At least one surface of the cantilever is coated with a capture layer, which capture layer reacts with a target molecule of interest. If the cantilever is exposed to a sample in

25 which the target molecule is present, the target molecule will react with the capture molecule on the cantilever surface and a surface stress change will be generated.

Due to the surface stress change of the cantilever, a

30 mechanical compression, stretch or decompression is applied to the cantilever and thereby also to the piezoresistor, and thereby the resistivity of the piezoresistor changes its value. The mechanical compression or decompression may result in a deflection

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and/or a stretch. By measuring the change in resistance, it can be determined whether the target molecule is present in the sample or not, and if so it may also be possibly to detect the concentration of the target molecule.

Cantilever-based sensors with integrated piezoresistive read-out are described by Thaysen, Ph.D. Thesis, "Cantilever for Bio-Chemical Sensing Integrated in a Microliquid Handling System", September 2001, Microelektronik Centeret, Technical University of Denmark. Hereby the stress changes on the cantilever sensors can be measured directly by the piezoresistor. Moreover, integrated read-out greatly facilitates operation in solutions since the refractive indices of the liquids do not influence the detection as it will using optical read-out. Each sensor may have a built-in reference cantilever, which makes it possible to subtract background drift directly in the measurement. The two cantilevers may be connected in a Whetstone bridge, and the stress change on the measurement cantilever is detected as the output voltage from the Whetstone bridge.

The objective of the present invention is to provide a sensor comprising one or more sensor units with a capture surface, which sensor can be used for detection of the presence of a target in a liquid, such as a biocomponent in a liquid with an improved signal or signal/noise ratio than according to the prior art cantilevered liquid sensors.

This and other objectives as it will be clear from the following description, has been solved by the invention as it is defined in the claims.

Disclosure of the invention

The sensor of the invention comprises one or more sensor units. The shape and size of the sensor and the size, shape and the number of sensor units as well as its wiring, may e.g. be as disclosed in any one of the patent applications WO 0066266, DK PA 2001 01724 DK PA 2002 00283, DK PA 2002 00125 and DK PA 2002 00195, which with respect to the disclosure concerning structure (shape and size of the sensor and the size, shape and the number of sensor units as well as its wiring) are hereby incorporated by reference.

In the following the sensor is described with one sensor unit, only, but it should be understood that the sensor unit may have several sensor units, such as up to 300, e.g. up to 100.

The sensor unit may in principle have any cantileverlike shape e.g. as the cantilevers described in DK PA 2002 00125. The term "cantilever shape" is defined as a sheet formed unit linked to a substrate (or two substrates) along one or two opposite edge lines. The term "cantilever shape" thus also includes a bridge, as well as a traditional rectangular or leaf shaped cantilever.

In one embodiment, the sensor unit shaped as a cantilever with a longitudinal direction is linked in both of its longitudinal endings to form a cantilevered bridge.

In another embodiment, the cantilever is a traditional rectangular or leaf shaped cantilever linked to only one

substrate. In the following this type of cantilevers are referred to as cantilevers with a free end.

5 The sensor unit comprises two major surfaces, wherein one or both of these totally or partly may constitute a capture surface.

10 In one embodiment the sensor unit is a flexible sheet-formed unit having an average thickness which is thinner than both its average length and its average width, said sensor unit preferably have a thickness of between 0.05 and 5 μm , such as between 0.2 and 4 μm , such as between 1 and 3 μm .

15 In one embodiment the sensor unit is a flexible sheet-formed unit having an average thickness which is at least 5 times, preferably at least 50 times less than its average width and average length.

20 The sensor unit has a capture surface e.g. in the form of a capture coating. The capture coating may e.g. be as described in any one of the applications DK PA 2002 00283 and DK PA 2002 00125 or in US 6289717, WO 0133226 or WO 0014539, which with respect to the disclosure concerning
25 the capture surface are hereby incorporated by reference.

30 In one embodiment of the sensor according to the invention, the capture surface is a surface of a capture coating comprising a capture layer, wherein said capture layer is a layer comprising a detection ligand, said detection ligand being a member of a specific binding pair wherein said detection ligand preferably is selected from the group consisting of RNA oligos, DNA oligos, PNA oligos, proteins, peptides, hormones; blood components,
35 antigen and antibodies.

The capture coating could in principle have any thickness. If the capture coating is very thick the sensitivity may be reduced due to stiffness of sensor unit. A desired thickness could e.g. be from molecular thickness to 2000 nm, such as up to, 2, 5, 10 or 50 molecule layers, or e.g. between 0.5 nm and 1000 nm, such as between 1 and 500 nm, such as between 10 and 200 nm.

- 10 In one embodiment both or a part of both of the two major sides of the cantilever comprise a capture surface. The capture surfaces may be identical or they may differ from each other e.g. with respect to size of area covered, type of capture molecules and/or concentrations thereof.
- 15 In one embodiment the capture surface on one major side of a cantilever is essentially identical, - both with respect to size of area covered, type of capture molecules and concentrations - to the capture surface on the other one of the two opposite major surfaces of the
- 20 cantilever. In this situation the stress generated on the cantilever when subjected to a liquid containing the target molecules, will be equal on both sides of the cantilever, and consequently, if the cantilever is of the type with a free end, the cantilever will not bend, but
- 25 only stretch.

In practice it is very cumbersome to produce a cantilever with to opposite major sides with identical capture surfaces. Thus, the cantilever will in most situations, even when carrying capture surfaces on both of each major sides, be subjected to at least a slightly bending due to different stress generated on the opposite major sides of the cantilever.

The sensor unit has a longitudinal direction and a transverse direction perpendicular to each other. The transverse direction of the cantilever is determined as the direction of the longitudinal middle line through the cantilever from the substrate to the free end of the cantilever or from one substrate to the other substrate. The transverse direction is perpendicular to the longitudinal direction and is determined as the direction of the transverse middle line through the cantilever.

The piezoresistive element is of an anisotropic material, and is arranged so that the numerically value of the sum of the longitudinal piezoresistive coefficient π_l and the transverse piezoresistive coefficient π_t is at least $10 \times P$, such as $20 \times P$, such as $30 \times P$, such as $40 \times P$, wherein P is the piezoresistance factor.

The piezoresistance factor P is depending on the doping level. P is between 0 and 1. For single crystalline silicon P is about 1 at a doping level around 10^{18} . Further information concerning the P factor and the determination thereof can be found in M. Tortonesi, H. Yamada, R.C. Barret and C. F. Quate. Atomic force microscopy using a piezoresistive cantilever. International Conference on Solid-State Sensors and Actuators. Digest of Technical Papers, page 448-451, 1991.

A piezoresistive effect in a material indicates the fractional change in bulk resistivity induced by a small mechanical stress applied to the material. Single crystalline silicon has a high piezoresistivity, and combined with its excellent mechanical and electronic properties, it makes it a useful material for the conversion of a mechanical signal into an electrical signal.

The piezoresistive element may therefore preferably be of doped single crystalline silicon. In one embodiment the piezoresistive element is of single crystalline silicon doped with 10^{16} ions/cm³ or more, such as 10^{17} ions/cm³ or more, such as 10^{18} ions/cm³ or more, such as 10^{19} ions/cm³ or more, such as 10^{20} ions/cm³ or more.

In one embodiment the piezoresistive element is of single crystalline silicon doped with 10^{20} ions/cm³ or less, such as 10^{19} ions/cm³ or less, such as 10^{18} ions/cm³ or less, such as 10^{17} ions/cm³ or less.

The higher level of doping ions the lower is the amount of noise, however simultaneously the signal will also be reduced accordingly. The temperature may also influence the noise as well as the signal, and accordingly the effect of temperature should also be considered. The optimal doping level can easily be found by the skilled person based on the present teaching. Optimal doping level will be in the interval 10^{16} ions/cm³ - 10^{20} ions/cm³.

In one embodiment the surplus or shortage of electrons due to the doping is within the interval 10^{16} ions/cm³ - 10^{20} ions/cm³.

The doping ions may in principle be any type of ions usable for doping silicon or mixtures of ions. In one embodiment, when n-type and p-type ions are mixed, it is desired that the piezoresistive element comprise at least 10^{16} ions/cm³, such as 10^{17} ions/cm³ or more, such as 10^{18} ions/cm³ or more, such as 10^{19} ions/cm³ or more, such as 10^{20} ions/cm³ or more, more of one of the types than of the other one of the types.

In one embodiment, the piezoresistive element being of single crystalline silicon doped with one or more of the ions boron ion, phosphorous ion, arsenic ion.

5 In one embodiment the piezoresistive element is n-type single crystalline silicon. The n-type single crystalline silicon may e.g. be orientated along the <110> direction of silicon. In another embodiment the n-type silicon piezoresistive element is orientated along the <100>
10 direction of silicon.

The piezoresistor may have any shape e.g. as described in any one of the patent applications WO 0066266, DK PA 01724 DK PA 2002 00283, DK PA 2002 00125 and DK PA 2002
15 00195. The piezoresistive element may e.g. be U shaped or V shaped.

The thickness of the piezoresistive element may e.g. be at least 10 nm, such as in the interval of 10 nm to 500
20 nm, such as in the interval of 50 nm to 300 nm, such as in the interval of 100 nm to 200 nm.

The sensor unit also comprise a pair of wires for applying an electrical field over the piezoresistor, e.g.
25 as described in any one of the patent applications WO 0066266, DK PA 01724 DK PA 2002 00283, DK PA 2002 00125 and DK PA 2002 00195.

In one embodiment of the sensor according to the
30 invention, where the sensor unit comprises two major surfaces, and at least a part of one or both of the major surfaces constitutes the capture surface, the piezoresistive element has a neutral plan distance of 50 nm or less, such as 100 nm or less, such as 200 nm or
35 less, such as 400 nm or less. The neutral plan distance is measured as the shortest distance between the middle

plan of the piezoresistive element and the neutral plan. The middle plan of the piezoresistive element is defined as the middle plan through the piezoresistive element which is parallel to the neutral plan. The neutral plan is defined as the plan along which the sum of the compressive and tensile stress acting on the piezoresistive element is as close to zero as possible.

The sensor unit further comprise a current shield, e.g. as described in DK PA 2002 00884 DK filed June 7, 2002.

The shield may have a diffusion barrier which is sufficient to prevent the diffusion of an electrolyte to leak current from the piezoresistor when an acidic liquid at a pH of 4 is held in contact with the capture surface for a period of 1 or even 2 minutes or even 10 minutes under standard conditions.

In one embodiment the shield is of a non-conducting material selected from the group consisting of nitrides, such as silicon nitride and tantalum nitride, non-conducting polymers, such as octafunctional epoxidized novalac, metal oxides, such as aluminium oxide, ceramics, diamond films, silicon carbide, tantalum oxide, silicon, glass, mixtures and combinations thereof.

In one embodiment the sensor unit comprise a bottom shield layer and a top shield layer, and an edge shield layer. The bottom shield layer, top shield layer and edge shield layer constitute the shield.

The sensor may preferably comprise one or more liquid chambers. In one embodiment the one or more sensor units partly or totally protrudes into the liquid chamber(s) so

10

that a liquid applied in the chamber is capable of coming into contact with part of the surface of the sensor unit(s).

- 5 The liquid chamber or chambers may e.g. be in the form of interaction chamber(s), preferably comprising a channel for feeding a liquid into the interaction chamber(s).

- 10 In one embodiment at least 50 %, more preferably substantially all of the capture surface of the sensor unit or units is positioned inside the liquid interaction chamber(s).

- 15 The sensor may e.g. be prepared as described in DK PA 2002 00884 DK with the difference that the doping may be an n-type doping and that the single crystalline silicon piezoresistive element is arranged so that the numerically value of the sum of the longitudinal piezoresistive coefficient π_l and the transverse piezoresistive coefficient π_t being at least $10 \times P$, such as $20 \times P$, such as $30 \times P$, such as $40 \times P$, wherein P is the piezoresistance factor.

Figures and examples

- 25 Embodiments of the invention will be described further with reference to the figures and examples.

- 30 Figure 1 shows the piezoresistive coefficients for π_l and π_t for p-type (A) and n-type (B) silicon. (At room temperature, in 10^{-11} Pa^{-1}).

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Figure 2 shows a Wheatstone bridge for converting the relative change in resistance from the measurement cantilever into a change in voltage.

5 Figure 3, shows a change in signal due to immobilization of 1mM Mercaptohexanol for respectively a sensor unit with an n-type piezoresistive element and a sensor unit with a p-type piezoresistive element. It can be seen that
10 the signal from the n-type cantilever is about a factor of 8 larger than the signal obtained from the p-type piezoresistive cantilever. Note that the change in output voltage for the n-type piezoresistive cantilever is negative compared to the p-type piezoresistive cantilever.

15 The piezoresistivity in single crystalline silicon is anisotropic and therefore the sensitivity is also dependent on the piezoresistor orientation with respect to the silicon crystalline. According to the invention it
20 has been found that the contribution to the relative resistance changes from stress generated on a cantilever surface is given by:

$$\frac{\Delta R}{R} = \sigma_l \pi_l + \sigma_t \pi_t \quad (1)$$

25

where σ_l and σ_t is the longitudinal and transverse stress respectively, while π_l and π_t indicates the piezoresistive coefficients. For p-type/n-type silicon wafer with (100) plane at the surface of the wafer the piezoresistive
30 coefficients at room temperature (in 10^{-11} Pa^{-1}) and doping level about 10^{18} cm^{-3} (p is approximately 1) is given in table 1.

12

	Direct ion	π_{11}	π_{12}	π_{44}	π_t	π_l
p-	<100>				0	0
Si	<110>	6.6	-1.1	138.1	-66	72
n-	<100>				53	102
Si	<110>	-102.2	53.4	-13.6	-18	-31

Table 1

The longitudinal piezoresistive coefficient in the <110> direction is determined as

5

$$\pi_l = 1/2 (\pi_{11} + \pi_{12} + \pi_{44}),$$

and the corresponding transverse coefficient is

10
$$\pi_t = 1/2 (\pi_{11} + \pi_{12} - \pi_{44}).$$

Figure 1 shows the piezoresistive coefficients for π_t and π_l for p-type (A) and n-type (B) silicon. It is seen that
15 the n-type piezoresistive coefficients are much more non-symmetrical than the p-type coefficients.

When a cantilever with integrated piezoresistor is used
20 as a longitudinally deflection sensor only, the piezoresistor is placed such that only the longitudinal stress is picked up. Since the cantilevers are usually released by a wet etch, such as KOH, the cantilever may
e.g. be aligned to the substrate such that a good clamping is obtained. Usually, the wet etch is
25 anisotropically and etches the <111> direction much slower than the other directions. Since the intersection of the {111} planes with the {100} are mutually perpendicular and lying along the <110> orientations, it

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is often desired to align the cantilever in the $\langle 110 \rangle$ direction in order to release it with a good clamping.

According to the invention it has been found that the surface stress generated on a sensor unit comprising a capture surface introduces a constant curvature or stretch at the areas where the surface stress is applied and no bending at places the surface stress is not applied. The constant curvature has shown to be obtained for both the transversal and the longitudinal direction. The situation can be visualised by placing the cantilever on a sphere. Since the surface stress changed is observed as a relative change in the resistance it has been found that both the transverse and longitudinal stress has to be considered, and furthermore, it has been found that they can be considered equally, irrespectively of the width and length of the piezoresistive material. It has thus been found that an optimal signal or signal/noise ratio is obtained when the piezoresistive element is arranged so that the numerically value of the sum of the longitudinal piezoresistive coefficient π_l and the transverse piezoresistive coefficient π_t is at least $10 \times P$, such as $20 \times P$, such as $30 \times P$, such as $40 \times P$, wherein P is the piezoresistance factor.

25

Example 1

A simulation of the surface stress sensitivity for a cantilever with the following dimensions, and with the piezoresistor placed in the $\langle 110 \rangle$ direction has been performed.

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The single crystalline silicon piezoresistor was
 5 simulated with respectively, a p-type and an n-type
 resistor. The result is:

p-type

10 $\frac{\Delta R}{R}/\sigma_x = 2.97 \cdot 10^{-4} (N/m)^{-1}$

n-type

15 $\frac{\Delta R}{R}/\sigma_x = 2.83 \cdot 10^{-3} (N/m)^{-1}$

As seen from the simulation the sensitivity for an n-type
 single crystalline silicon piezoresistor used for surface
 stress sensitivity is almost a factor of 10 more
 sensitive than a p-type single crystalline silicon
 20 piezoresistor.

Cantilever Dimensions	Thickness [nm]	Young's modulus [GPa]	Pre-stress [MPa]
Au	30	80	40
SiN	45	200	85
Si	150	180	20
SiO2	100	70	-290
SiN	145	200	75

25 **Example 2**

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In order to exemplify the increase of sensitivity by using an n-type piezoresistive cantilever as a surface stress sensor instead of a p-type piezoresistive cantilever, an experiment that changes the surface stress
5 in a controlled manner is performed.

An example of such an experiment is measuring the change in surface stress when the molecule Mercaptohexanol is immobilized to a gold layer on the topside of a
10 cantilever surface. The immobilization of Mercaptohexanol is performed due to the binding between the -SH group in Mercaptohexanol and the gold layer. The immobilization of Mercaptohexanol is finalized when a complete monolayer is formed on the gold surface. Since the surface stress of
15 the cantilever is changed during this procedure, this can be monitored as a change in signal from the piezoresistive cantilever. When the monolayer has been formed the signal will become constant. The amplitude of the signal is then defined as difference between the
20 signal before the introduction of Mercaptohexanol and the signal from the piezoresistor after the Mercaptohexanol monolayer is formed on the cantilever gold.

The experiment consists of observing the amplitude of two
25 almost identical piezoresistive cantilevers, wherein the cantilevers differs in that one of the piezoresistive cantilevers has incorporated an n-type piezoresistor while the other has a p-type piezoresistor. In this example the piezoresistors is placed in the <110>
30 direction of the silicon crystal and P=1 for both n-type and p-type. The piezoresistors are inserted in a Wheatstone bridge as seen in figure 2. The change in output voltage from the Wheatstone bridge is given:

$$\Delta V = \frac{1}{4} \frac{\Delta R}{R} \sigma_1 \cdot V$$

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Where ΔV is the output voltage and V is the input voltage, σ_s is the surface stress and $\Delta R/R$ is the relative change of resistance of the piezoresistor. Since the relative change in resistance is given by:

$$\frac{\Delta R}{R} = \pi_l \cdot \sigma_l + \pi_t \cdot \sigma_t$$

Where π_l and π_t are the piezoresistive coefficients in the longitudinal and transversal direction, respectively. σ_l and σ_t are the stress in the piezoresistive layer. It can be assumed that $\sigma_l = \sigma_t = \sigma$ and $\sigma_t \propto \sigma$.

Using the above considerations together with the piezoresistive coefficients in the table 1, the output voltage for a p-type piezoresistive cantilever can be written as:

$$\Delta V_{p-type} \propto (\pi_l + \pi_t) \sigma_s \cdot V = 6 \cdot \sigma_s \cdot V$$

and for an n-type piezoresistor:

$$\Delta V_{n-type} \propto (\pi_l + \pi_t) \sigma_s \cdot V = -49 \cdot \sigma_s \cdot V$$

As it is seen from the two equations, there is a factor of about 8 in difference in sensitivity in favour of n-type piezoresistive cantilever, and the change in output voltage has opposite signs for the same surface stress.

In the experiment, the cantilevers are inserted in a micro liquid handling system as described in WO 0066266. The $V=2.5$ V is applied to the Wheatstone bridge (input voltage) and the signal from the Wheatstone bridge is

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monitored by a voltmeter. First, water is pumped through the system in order to stabilize the system. Hereafter, 1 mM Mercaptohexanol is introduced in the micro liquid handling system and the signal starts to change. The graph in figure 3 is an example of such an experiment. It can be seen that the signal from the n-type piezoresistive cantilever has a signal, which is about 8 times larger, and with opposite sign compared to the change in signal from the p-type piezoresistive cantilever.

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Patent Claims:

1. A sensor comprising at least one sensor unit shaped as a cantilever with a longitudinal direction and a transverse direction perpendicular to each other, said sensor unit comprises a capture surface, and a piezoresistive element extending in a longitudinal direction and a transverse direction perpendicular to each other, said sensor further comprises pair of wires for applying an electrical field over the piezoresistive element, and said piezoresistive element being of an anisotropic material, and being arranged so that the numerically value of the sum of the longitudinal piezoresistive coefficient π_l and the transverse piezoresistive coefficient π_t being at least $10 \times P$, such as $20 \times P$, such as $30 \times P$, such as $40 \times P$, wherein P is the piezoresistance factor.
2. A sensor according to claim 1 wherein the piezoresistive element being of doped single crystalline silicon.
3. A sensor according to any one of the claims 1 and 2 wherein the sensor unit comprises a single crystalline silicon piezoresistive element encapsulated in a single crystalline silicon electrically shield.
4. A sensor according to any one of the preceding claims wherein said sensor unit shaped as a cantilever with a longitudinal direction is linked in both of its longitudinal endings to form a cantilevered bridge.
5. A sensor according to claim 4 wherein the sensor unit has a thickness in the interval of $0.05 \mu\text{m}$ to $5 \mu\text{m}$, such as in the interval of $0.2 \mu\text{m}$ to $4 \mu\text{m}$, such as in the interval of $1 \mu\text{m}$ to $3 \mu\text{m}$.

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6. A sensor according to claim 4 wherein the piezoresistive element has a thickness in the interval of 10 urn to 500 nm, such as in the interval of 50 nm to 300 nm, such as in the interval of 100 nm to 200 nm.

7. A sensor according to any one of the preceding claims wherein the piezoresistive element being U shaped or V shaped.

8. A sensor according to any one of the preceding claims wherein the piezoresistive element is n-type single crystalline silicon.

9. A sensor according to claim 8 wherein said n-type silicon piezoresistive element being orientated along the <110> direction of silicon.

10. A sensor according to claim 8 wherein said n-type silicon piezoresistive element being orientated along the <100> direction of silicon.

11. A sensor according to any one of the preceding claims wherein the piezoresistive element being of single crystalline silicon doped with one or more of the ions: boron ion, phosphorous ion, arsenic ion.

12. A sensor according to any one of the preceding claims wherein the piezoresistive element being of single crystalline silicon doped with 10^{16} ions/cm³ or more, such as 10^{17} ions/cm³ or more, such as 10^{18} ions/cm³ or more, such as 10^{19} ions/cm³ or more, such as 10^{20} ions/cm³ or more.

13. A sensor according to any one of the preceding claims wherein the piezoresistive element being of single

crystalline silicon doped with 10^{20} ions/cm³ or less, such as 10^{19} ions/cm³ or less, such as 10^{18} ions/cm³ or less, such as 10^{17} ions/cm³ or less.

5 14. A sensor according to any one of the preceding claims, wherein the sensor unit comprises two major surfaces, and at least a part of one or both of the major surfaces constitutes the capture surface, and the piezoresistive element has a neutral plan distance of 50
10 nm or less, such as 100 nm or less, such as 200 nm or less, such as 400 nm or less, which neutral plan distance is measured as the shortest distance between the middle plan of the piezoresistive element, defined as the middle plan through the piezoresistive element which is parallel
15 to the neutral plan, and the neutral plan, which neutral plan is defined as the plan along which the sum of the compressive and tensile stress acting on the piezoresistive element is as close to zero as possible.

20 15. A sensor according to any one of the preceding claims, wherein the sensor unit comprises two major surfaces, which major surfaces partly or totally constitute a capture surface.

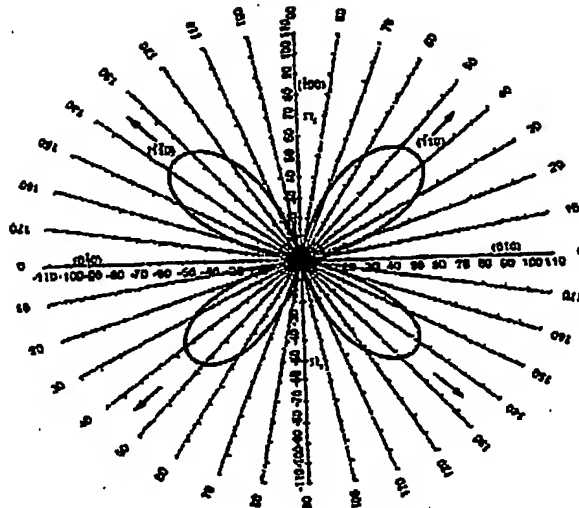
25 16. A sensor according to any one of the preceding claims wherein said sensor comprises one or more liquid chambers, said one or more sensor units partly or totally protrudes into said liquid chamber(s) so that a liquid applied in the chamber is capable of coming into contact
30 with part of the surface of the sensor unit(s).

17. A sensor according to any one of the preceding claims wherein said liquid chamber or chambers is/are in the form of interaction chamber(s), preferably comprising
35 a channel for feeding a liquid into the interaction chamber(s).

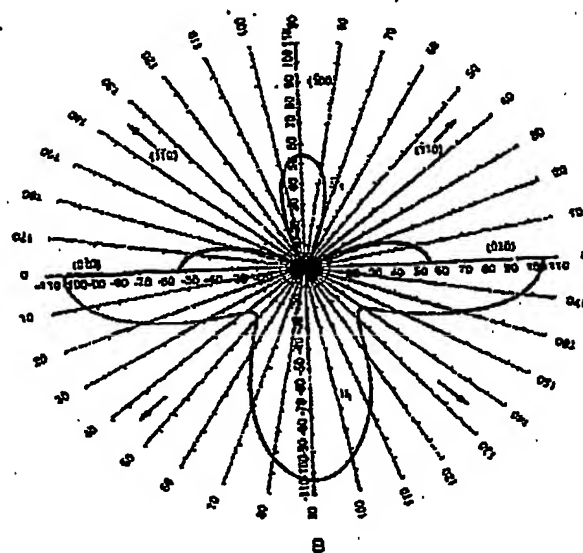
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Figure 1



A



B

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Figure 2

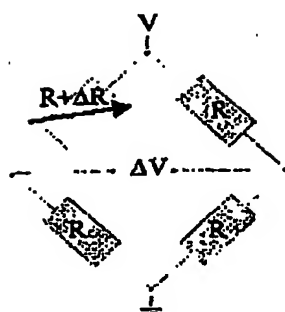
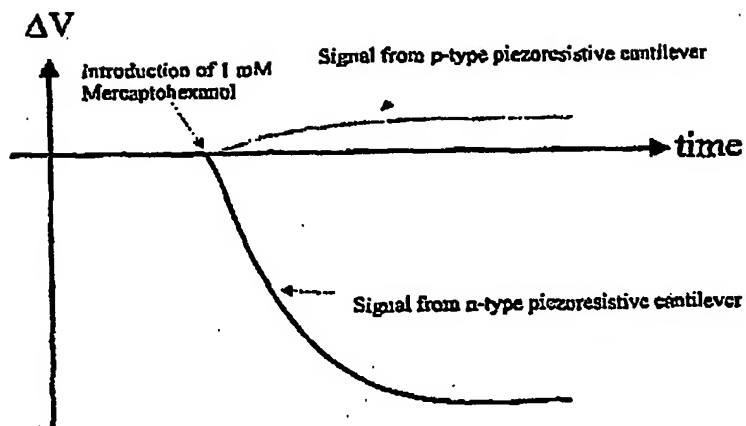


Figure 3



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